Scientists recently reported an analgesic compound in frog skin that is 200 times more powerful than morphine.

FROGS AND DRUGS

BY MICHAEL J. TYLER

THE ROMAN PLINIUS SECUNDUS (Pliny to his friends) and the Englishman, Francis Bacon, have both been described as "martyrs to experimental science". For Bacon died of a chill that he caught while stuffing a chicken with snow, in order to test the preservative properties of refrigeration, and Pliny was asphyxiated by volcanic gases while investigating the eruption of Mount Versuvius in AD 79.

For hundreds of years, folk medicine has known of the medicinal value of frogs. Certain groups of Gipsies, for example, relieved joint pain with the fresh spawn of grass frogs (*Rana* sp.).

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Bacon and Pliny were equally fascinated by folk medicine, and the latter solemnly and unquestioningly recorded every treatment he heard about. Some were revolting and others absurd. For example, digging up the soil where a dog had just urinated and applying this to a joint seems an unlikely treatment for a dislocation. Similarly, the prospect of curing jaundice by staring at a canary, or ensuring that a baby will have black eyes by inducing a pregnant woman to eat a rat are, at best, unconventional. Not surprisingly, folk medicine fell into disrepute.

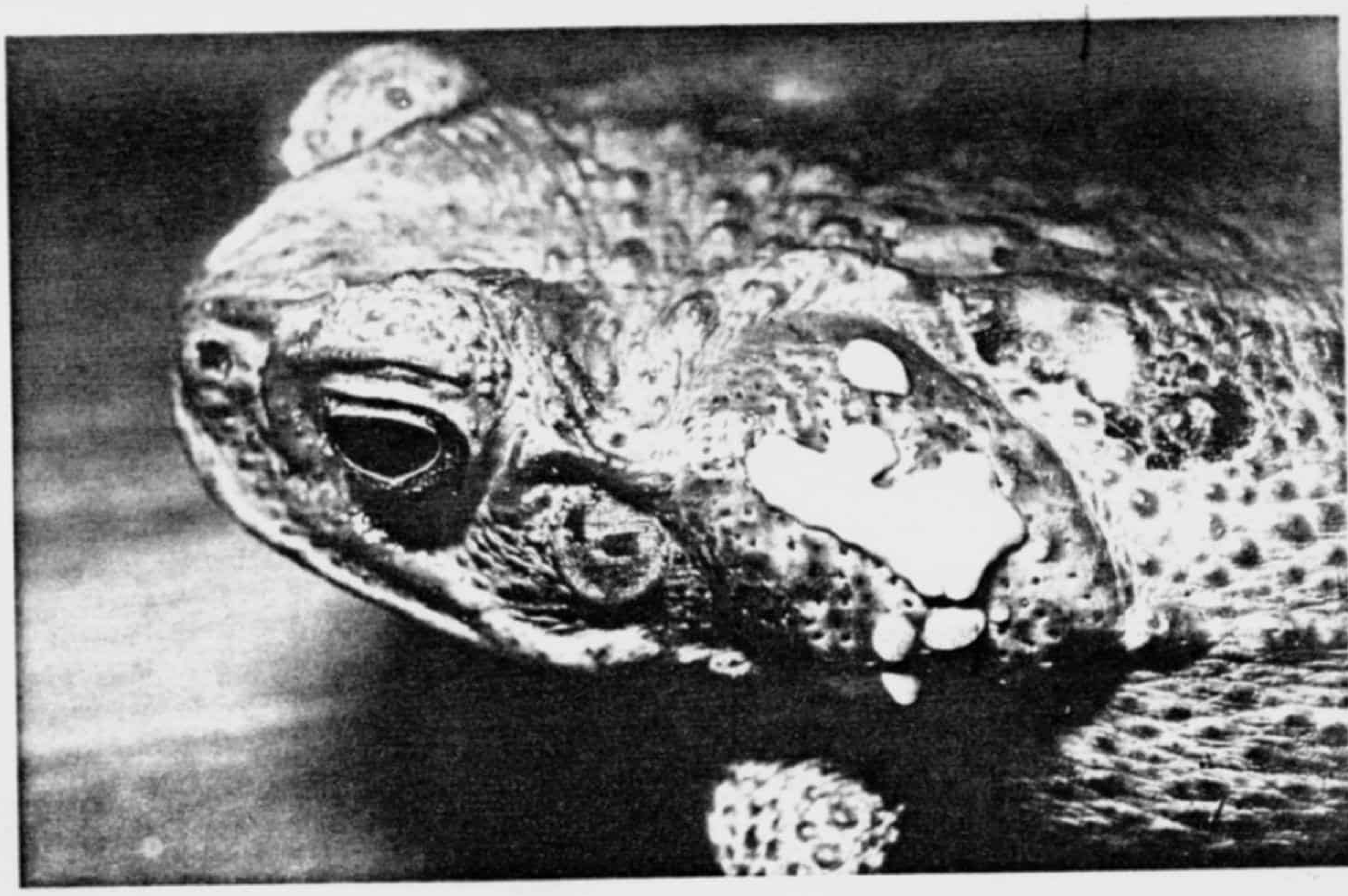
But now there is a resurgence of interest in the so-called 'alternative medicines', and frogs and toads are of special interest because they have a wide array of compounds of clinical potential in their skin. In fact, their skin is more complex than that of any other type of living creature. Scattered throughout the outer layer of the upper surface are circular glands that produce a variety of chemical compounds. For example, there are mucous glands that keep the surface slightly moist for gas exchange and for cooling by evaporation. There are also lipid (fat) secreting glands that provide frogs with a waterproofing agent. Nerves in the deeper layer of the skin trigger the release of all these skin chemicals.

The glands that are of current interest are termed granular glands and these are filled with circular vesicles a few microns (millionths of a metre) across. It is these vesicles that contain the chemicals. Sometimes the granular glands are aggregated together into giant superglands like those on the shoulders of the Cane Toad (*Bufo marinus*), or upon the entire head of the Magnificent Tree Frog (*Litoria splendida*). There may be 20 or 30 chemical compounds in a single species and every species seems to have at least some chemicals not possessed by others.

The granular gland secretions of some species can be highly toxic, such as those belonging to the South and Central American poison-dart frogs (family Dendrobatidae). These are so named because Colombian Indians tipped their darts with frogs' skin secretions and, using a long blowpipe, were able to capture birds and small mammals. The frogs are often brightly coloured, presumably a warning to creatures that might eat them. Three major groups of toxins have been discovered in their skin: batrachotoxins, pumiliotoxins and histrionicotoxins. The toxins are as complex as their names and include compounds that are far more virulent than the most venomous snake; in fact they have been described as among the most toxic substances known to mankind.

Not all frog and toad secretions are toxic. In 1757 Oliver Goldsmith wrote of an amorous woman of Rome married to an invalid husband who suffered from a weak heart and fluid retention. Wishing to hasten her husband's death she made a potion from a toad and slipped it into her husband's drink. But she made a fundamental error in the identity of the toad species. The one she selected contained a heart stimulant and a diuretic, which increased the flow of urine. The result was that she cured the husband she detested and restored him to perfect health.

In general, however, toads are poisonous, and not just the skin glands. A university student in Sydney recently discovered this when, for a bet, he ate the ovaries of a Cane Toad. He received $20 and a cardiac arrest.



JIM FRAZIER/MANTIS WILDLIFE FILMS

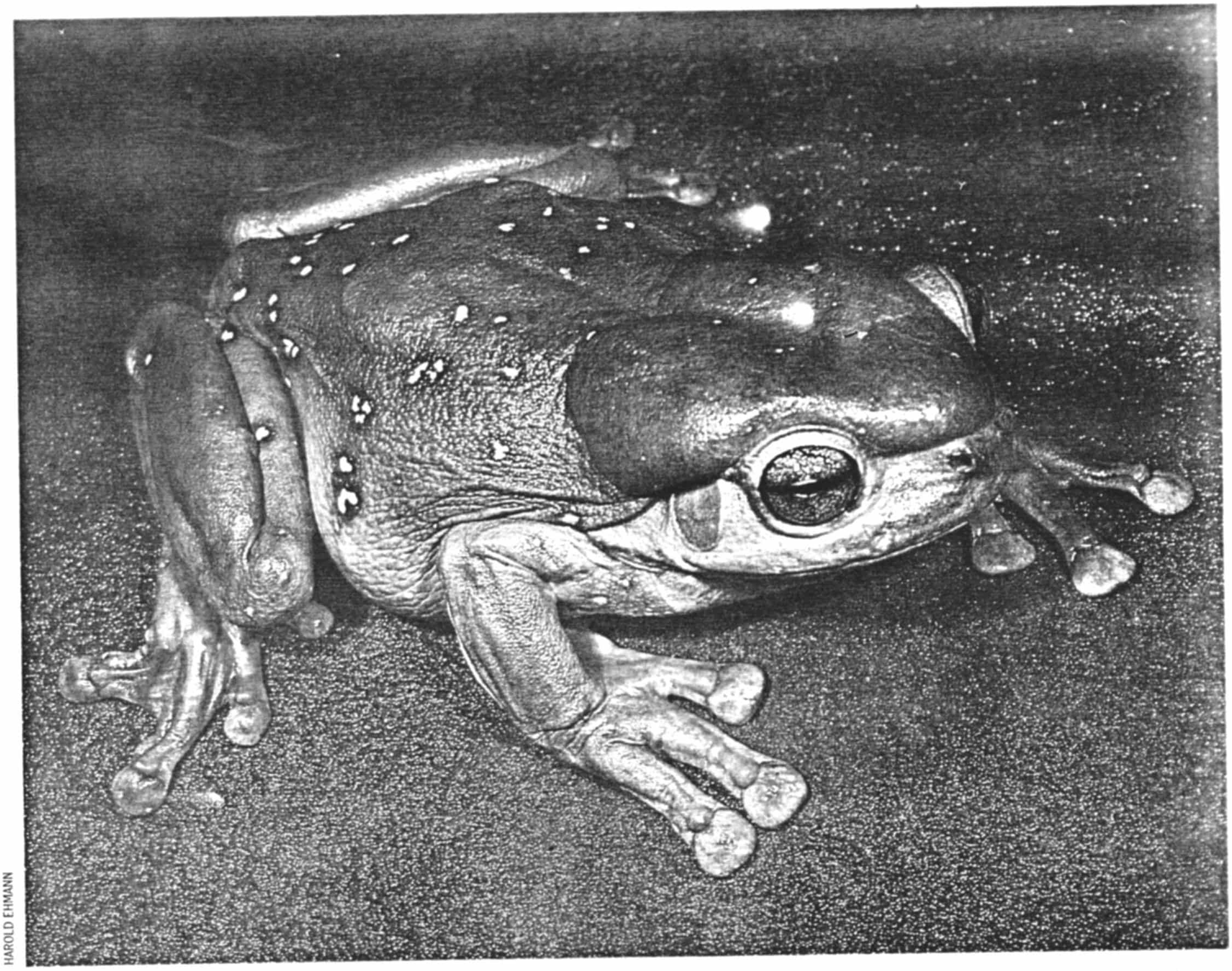
The granular glands of Cane Toads are grouped on their shoulders to form super-glands. These superglands exude a poisonous creamy substance used by the frog for defence but which may contain a number of compounds valuable to humans.

The skin of the Green Tree Frog (shown here in its blue form) produces a compound which can reduce blood pressure and restore gut function after surgery .

H. & J. BESTE/NATURE FOCUS

MICHAEL FOGDEN/DRK PHOTOS

The granular gland secretions of poison-dart frogs include some of the most toxic substances known. These male Strawberry Poison-dart Frogs (*Dendrobates pumilio*) from Costa Rica are wrestling over territory.



HAROLD EHMANN

The supergland on the head of the Magnificent Tree Frog exudes a cocktail of chemicals, including some with antibiotic properties.

Occasionally just a hunch can lead to a great discovery. Thus in 1986, Professor Michael Zasloff of the National Institute of Health in the USA became curious at the fact that the common laboratory African Clawed Frog (*Xenopus laevis*) never suffered from infections following experimental surgery. He correctly forecast the existence of antibiotics in the skin, and an American pharmaceutical company is now developing them for human use.

A totally different compound was found in the skin of the Green Tree Frog (*Litoria caerulea*) of northern Australia. Named caerulein in honour of the frog, it has been marketed in Germany under the brand name of Ceruletid. Tested initially as a drug to reduce high blood pressure, it was found to be a powerful stimulant of the gut and has found a role in restoring gut movements following major abdominal surgery. It has also been put to good use in the human brain, with symptoms of chronic schizophrenia being blocked for a month following a single injection.

Pain-killing compounds resembling opiates have been found in the skin of *Phyllomedusa* species of South America, and a group of scientists in Baltimore recently reported an analgesic compound in frog skin that is 200 times more powerful than morphine.

I BECAME INVOLVED IN THE STUDY OF FROG skin secretions in the 1960s but it was only in 1977 that it became possible to identify them. I talked to Professor John Bowie of the Department of Chemistry at the University of Adelaide, and we formed a research team to isolate the various ingredients of the skin cocktails.

The trigger for our work was the discovery of the Magnificent Tree Frog (see ANH Autumn 1994). I was simply fascinated by the huge gland on its head, and wanted to know what it contained. When I first handled one of these frogs, it exuded a creamy secretion that smelt like cashew nuts.

An initial problem was to find a means of inducing the Magnificent Tree Frog to release its secretions, because in captivity this species, like its close relative the Green Tree Frog, settles down very quickly, is happy to be handled, and simply will not release secretions for an investigator. I was not prepared to kill such fantastic creatures and remove and mash their skins just to look at their skin secretions. There had to be an alternative. One day, while having acupuncture to relieve headaches, I was idly watching one pair of needles inserted into the skin between the base of my thumb and forefinger. The needles flicked once each second as muscles beneath the skin received electric shocks from a battery-powered unit nearby. Because the stimulus was so mild and localised, but effective, I wondered whether the muscles in the frog skin could be made to contract in the same way, causing the glands to liberate their secretions. That weekend I returned to the acupuncturist's rooms with a cathode ray oscilloscope and measured the electrical characteristics of his instrument. A manufacturer of acupuncture stimulators in Melbourne agreed to modify one of his units for me. When it arrived in Adelaide I took a frog and gently rubbed its back with a pair of silver electrodes (instead of sticking needles into its skin). For ten seconds nothing happened and I worried that I had made a great mistake,

The skin of certain South American *Phyllomedusa* frogs produce pain-killing compounds.

but then the secretions poured out and could be washed off into a container with a jet of distilled water. The frogs gave no indication of enjoying the sensation, but I am comforted by the response of Maurice Chevalier who, after a lifetime of entertaining the world, was asked whether he enjoyed old age. "No", he replied, "but the alternative is worse".

From a single milking we could obtain up to 100 milligrams of crude extract. Microbiologists tested the extracts upon cultures of bacteria, fungi and viruses, and demonstrated their ability to inhibit the growth of the virulent bacterium *Staphylococcus aureus* ('Golden Staph') and the virus *Herpes simplex*. The next step was to find out what effects the secretions had upon living mammalian cells. If they were toxic we could abandon any plans to explore them further because, obviously, they could not be used as future drugs. The results were favourable—the compounds did not harm the cells. But the crude secretions were a cocktail of as many as 30 chemicals and we did not know which of them had the antibiotic properties. So my chemist colleagues isolated and worked out the structural formula for each chemical and we took these to a biotechnology company, which manufactured some of them at a cost of $2,000–$3,000 for just ten milligrams per chemical. These were tested on a variety of bacteria, fungi and viruses, but the results were not always the same. It seems that a cocktail works better than one chemical alone. Clearly this research is expensive and currently we lack the support of a major drug company to test the remaining chemicals in the secretion. Without help I doubt if our findings will ever be exploited and brought to the pharmacy shelf.

Despite great interest in frog skin secretions, few species have been examined other than extremely superficially. With 4,000 species of frogs now known it makes sense to follow up the folklore, as a short cut in the selection process. And it is not only antibiotics that await investigation. Gipsies in Germany and Czechoslovakia relieved the pain and swelling of rheumatic joints by applying a poultice of fresh spawn of grass frogs (*Rana* species). Presumably there is an anti-inflammatory compound in the jelly.

In many countries, including Australia, people are alarmed at the decline and disappearance of frog populations (see ANH Autumn 1991). The discovery that frogs are mobile pharmacies may just provide the stimulus to assist their survival. ■

Further Reading

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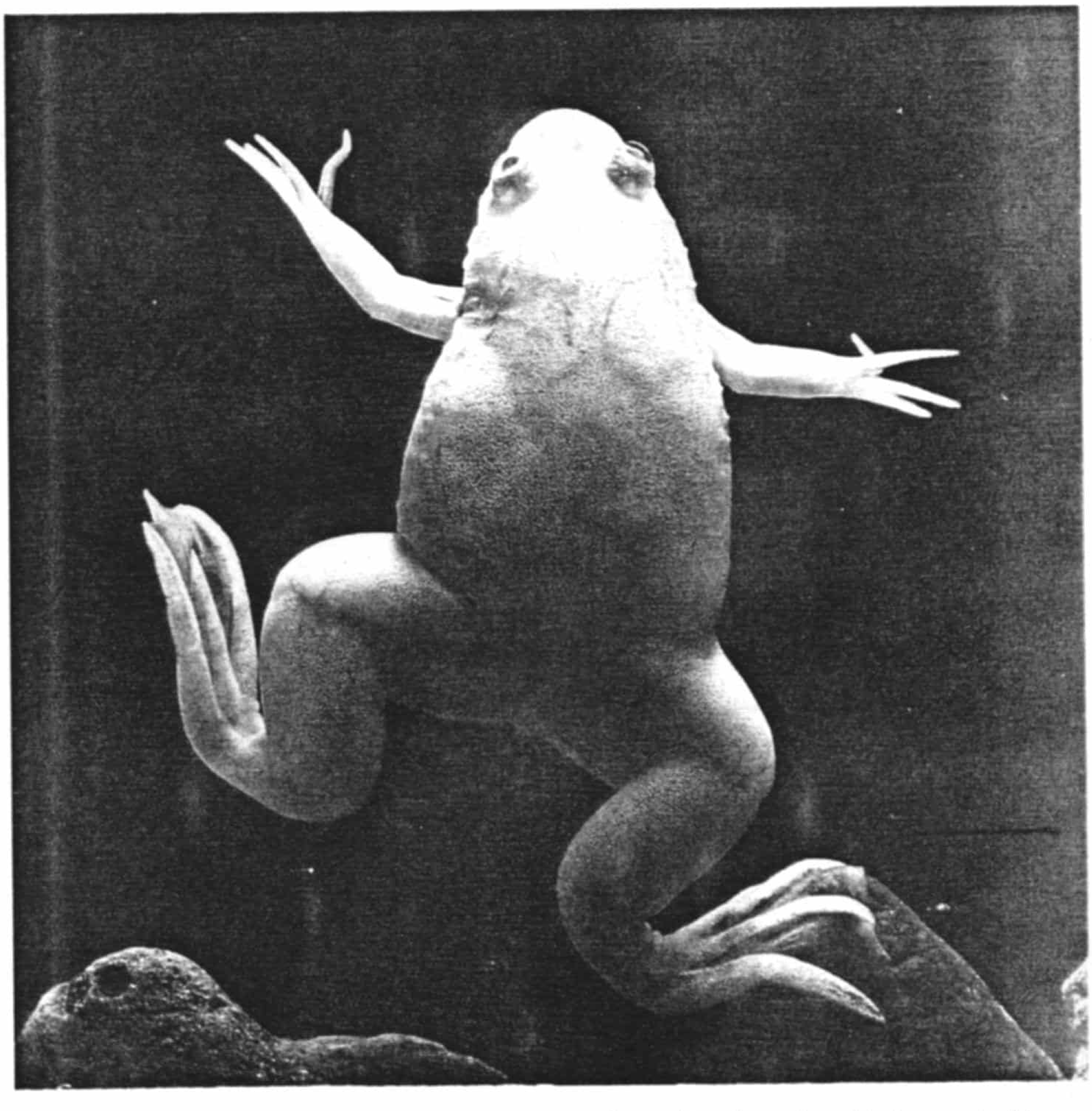

African Clawed Frogs, like this albino laboratory specimen, have been found to have valuable antibiotics in their skin.